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IgE Responsiveness to *Dermatophagoides farinae* in Asthma and Atopic Dermatitis

Key Words

Asthma
 Atopic dermatitis
 IgE antibody
Dermatophagoides farinae
 House dust mite
 Immunoblotting
 RAST
 Der f1
 Der f2

Abstract

Using Western blotting and RAST, this study examined IgE reactivities to *Dermatophagoides farinae* (Df) extract in three groups of children with either asthma, atopic dermatitis (AD) or a combination of both. The Df antigen presented at least nine distinct IgE-binding bands, with apparent molecular weights of 15, 25, 30, 40, 48, 59, 67, 80 and 110 kD. Positive IgE binding reactions to 15-kD proteins were found in more than 85% of subjects in all three groups, and positive reactions to 25-, 30- and 110-kD proteins in 35–45% in each group. The frequencies of positive IgE binding reaction to other components were generally low in all three groups. The pattern of IgE reactivity to Df components did not differ significantly between the three groups of subjects. IgE-binding activities to two major allergens, Der f1 and Der f2, were also examined by RAST, using recombinant antigens. High RAST reactivities to Der f1 and Der f2 were found in three groups, and the average RAST levels for Der f1 and Der f2 did not differ significantly between the three groups. These findings suggest that a difference in IgE responsiveness to house dust mite antigen is not a major discriminative factor in determining the difference between asthma and AD.

Introduction

Allergic sensitization to house dust mite (HDM) is believed to play an important role in the development of asthma in children because high levels of IgE antibody against the mite antigen show significant correlations not only with a clinical history of asthma, but also with a provocation test result with this antigen [1–4]. It has also been shown that patients with atopic dermatitis (AD) have elevated levels of IgE antibodies against HDM [5]. Positive immediate prick test and delayed cutaneous patch test reactivities were more prevalent in patients with AD than in control subjects, and avoidance of these allergens improved the symptoms of AD [6–8]. These findings suggest that HDM may be associated with the pathogenesis of AD [6, 8], as well as asthma.

HDM antigens are known to be heterogeneous, including more than 30 IgE-binding components [1, 9–18]. Some of these components of protein were isolated from body and fecal extracts of *Dermatophagoides farinae* (Df) or *Dermatophagoides pteronyssinus* [11–13], and defined as major allergens, including Der 1 and Der 2.

Thompson et al. [19] have reported using a small number of samples that patients with AD produce more IgE antibodies to Der p 2, and asthmatics produce more antibodies to Der p 1. This suggests that the difference in the variety of HDM antigenic materials that subjects are sensitized to may be an important discriminative factor separating asthma from AD. To further evaluate this problem, we compared the IgE reactivity to whole Df antigen between three groups of children with asthma, with AD or with a combination of

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both by Western blotting in a larger sample. In addition, IgE reactivities to two major allergens of Df, Der f1 and Der f2, were also compared between the three groups by RAST.

Materials and Methods

Antigen

Lyophilized mite body extract prepared from isolated mite bodies of Df was kindly supplied by Torii Co., Ltd. (Tokyo, Japan), and used as Df antigen [10]. The Der f1 and Der f2 contents of this antigen were estimated to be 0.53 and 0.48%, respectively. Recombinant Der f1 and Der f2 were kind gifts from Dr. Okumura of the Central Laboratory of Asahi Breweries (Tokyo, Japan). The recombinant allergens were shown to have the same antigenic activity as the native Der f1 and Der f2 [20].

Subjects

The subjects studied were children attending the Pediatric Allergy Clinic of the University Hospital of Tsukuba in Japan. The diagnosis of asthma was based on the definition of the American Thoracic Association [21], and patients with two or more episodes of wheezing with shortness of breath during the past year were considered as having asthma. AD was diagnosed based on the criteria established by Hanifin and Rajka [22] in 1980. A total of 270 patients participated in this study, and their Df-specific IgE antibodies were determined by the Sepharose RAST method as described below. The patients having a Df-RAST titer of 1:128 or more were first screened for IgE-binding studies. The high RAST patients were divided into three groups according to their clinical manifestations: asthma, AD or both asthma and AD (asthma+AD). Finally, 20 sera were randomly selected from each of the three groups. The average ages of children with asthma, AD and asthma+AD were 7.6 ± 4.5 , 6.7 ± 3.8 and 7.7 ± 4.7 years, their mean serum IgE levels were, $1,428 \pm 888$, $1,021 \pm 909$ and $1,190 \pm 915$ IU/ml, their average reciprocal RAST titers for Df $1,510 \pm 1,289$, $1,318 \pm 1,198$ and $1,280 \pm 813$, respectively.

Sepharose-RAST

The Sepharose-RAST method has been described in detail in a previous article [23]. One milligram of Df antigen and 50 μ g of Der f1 and Der f2 were coupled to 200 mg of CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the method described by Wide et al. [24].

The antigen-coupled particles (ACP) were suspended in RAST buffer obtained from Phadebas RAST kits (Pharmacia, Uppsala, Sweden) at a concentration of 20 mg/ml. Fifty microliters of ACP suspension were incubated for 3 h at room temperature with 50 μ l of sera. After washing 3 times with 4 ml of RAST buffer (0.15 mol/l of NaCl solution containing 0.1% Tween 20), 50 μ l of ^{125} I-labeled anti-IgE (25,000 cpm) obtained from Phadebas RAST kits (Pharmacia) were added to the tubes, and the mixture was further incubated for 16 h. Excess labeled anti-IgE was washed away with 0.15 mol/l of NaCl solution containing 0.1% Tween 20. Radioactivity bound to the ACP was measured in a gamma counter. RAST determinations were done in duplicates, and the mean value was used for calculation of the RAST binding. The RAST value was expressed as a % binding: the counts bound to ACP/the total counts added $\times 100$.

The cutoff point between RAST positivity and negativity was defined as 350% of the background binding produced by 2% HSA. The

results were regarded as positive when the percentage of sample serum binding was higher than the cutoff value. In order to verify the specificity of this assay, RAST determinations were performed on 30 sera from patients with allergic rhinitis who had shown positive skin test results to *Cryptomeria* and orchard grass, and negative skin test results to Df and two IgE myelomatous sera (2,000 IU/ml). None of the sera exceeded the cutoff value.

Measurement of RAST Titer

Serial 2-fold dilutions of sera were prepared and then subjected to RAST. The maximum dilution of serum producing RAST positivity was expressed as a RAST titer.

IgE Immunoblotting

SDA-PAGE was carried out as previously described [25]. A 90 $\times$ 40 mm minigel was used, and the sample gel was divided into two lanes by use of a special comb: one lane was 3.5 mm wide, for molecular weight marker standards (Calibration kit proteins, Pharmacia, Sweden), and the other 80 mm wide for sample application.

The gels were loaded with 200 μ g of protein for IgE blotting and electrophoresed at 30 mA per gel in the unreduced condition. After electrophoresis, gels were electroblotted onto a polyvinylidene difluoride membrane (Immobilon®, Millipore, Bedford, Mass., USA) in a Tris-glycine-SDS-methanol buffer at 0.8 mA/cm. After the transfer, the lane containing the molecular weight marker standards was removed and sequentially stained with amido black. The blotted samples were blocked for 3 h in PBS buffer containing 0.01% Tween 20 and 3% BSA. The blots were then washed 5 times in PBS-Tween and cut into 21 strips, each 3 mm wide. For immunoblotting the sera were first adjusted so each sample would have a RAST titer of 1:128. The sera were then diluted 1/10 with PBS buffer, and 1 ml of each diluted serum was incubated overnight with strips in special strip incubation tubes with 1 ml of test sera, washed again and incubated for 4 h with 0.2 μ Ci of ^{125} I-labeled antihuman IgE obtained from Phadebas RAST kits (Pharmacia). The strips were washed, dried and autoradiographed with a Fujix BAS2000 Imaging Analyzer (Fuji Photo Film, Tokyo, Japan).

The intensity of IgE-binding bands was graded as 0 to 11, referring to the sensitivity control gray scale for X-ray film (Wejtec, Iuyland, Penn., USA). When the intensity was grade 2 or higher, the reaction was defined as positive.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney test. The difference was considered significant at $p < 0.05$.

Results

Comparison of IgE Reactivity of Df by Immunoblotting

The immunoblot profiles of IgE reactivities to Df antigen in asthma, AD and asthma+AD groups are illustrated in figure 1A–C, respectively. As determined visually, the pattern of the IgE reactivity was not different between the three groups. The Df antigen presented at least nine distinct IgE-binding bands with molecular weights of 15, 25, 30, 40, 48, 59, 67, 80 and 110 kD. Sera from the three groups tended to bind low molecular weight proteins, less than 30 kD, and, especially, the 15- and 25-kD proteins. The frequencies of

positive IgE-binding reactions in asthma, AD and asthma+AD groups are summarized in figure 2A–C, respectively. In the asthma group, positive IgE-binding reactions to 15- and 25-kD components were found in 18 of 20 (90%) and 7 of 20 sera (35%), and those binding to 30 and 110 kD in 7 of 25 (35%) and 8 of 20 sera (40%), respectively. In the AD group, positive reactions to 15- and 25-kD components were found in 17 of 20 (85%) and 7 of 20 sera (35%), and to 30 and 110 kD in 7 of 20 (35%) and 9 of 20 sera (45%), respectively. In the asthma+AD group, IgE-binding reactions to 15-, 25-, 30- and 110-kD components were positive in 20 of 20 (100%), 10 of 20 (50%), 6 of 20 (30%) and 8 of 20 sera (40%), respectively. IgE-binding reactions to other components were very low in all three groups. Thus, the pattern of the IgE reactivity to Df components did not differ significantly between the three groups of subjects.

Comparison of RAST Reactivities to Der f1 and Der f2 between Three Groups of Children

The sample sera were adjusted to have the same RAST titer for Df (1:32), and relative RAST reactivities to Der f1 and Der f2 were compared between the three groups. The average RAST levels for Der f1 in asthma, AD and asthma+AD were 5.1 ± 4.0 , 4.4 ± 2.2 and $4.2 \pm 2.4\%$ binding, respectively, and positive RAST rates to Der f1 were found in 19 of 20 (95%) in all three groups (fig. 3). The average RAST levels for Der f2 in asthma, AD and asthma+AD were 9.4 ± 5.3 , 11.5 ± 4.5 and $9.7 \pm 4.7\%$ binding, and positive RAST rates to Der f2 were found in 19 of 20 (95%), 20 of 20 (100%) and 20 of 20 sera (100%), respectively (fig. 4). There was no significant difference in the RAST levels for Der f1 and Der f2 between the three groups ($p > 0.1$).

Discussion

The IgE immunoblot study with Df body extract demonstrates nine distinct IgE-binding bands with apparent molecular weights of 15, 25, 30, 40, 48, 59, 67, 80 and 110 kD. The pattern of IgE reactivity to Df components, with a dominant response to 15- and 25-kD proteins, did not differ significantly between the three groups: subjects with asthma, AD or asthma+AD. In addition, our RAST results have shown that both asthmatic and AD children have a high RAST reactivity to Der f1 and Der f2 (more than 95% positivity) and that relative RAST levels for Der f1 and Der f2 do not differ significantly between the three groups. These findings suggest that the difference in IgE responsiveness to HDM is not a major discriminative factor for determining the clinical manifestation of asthma or AD.

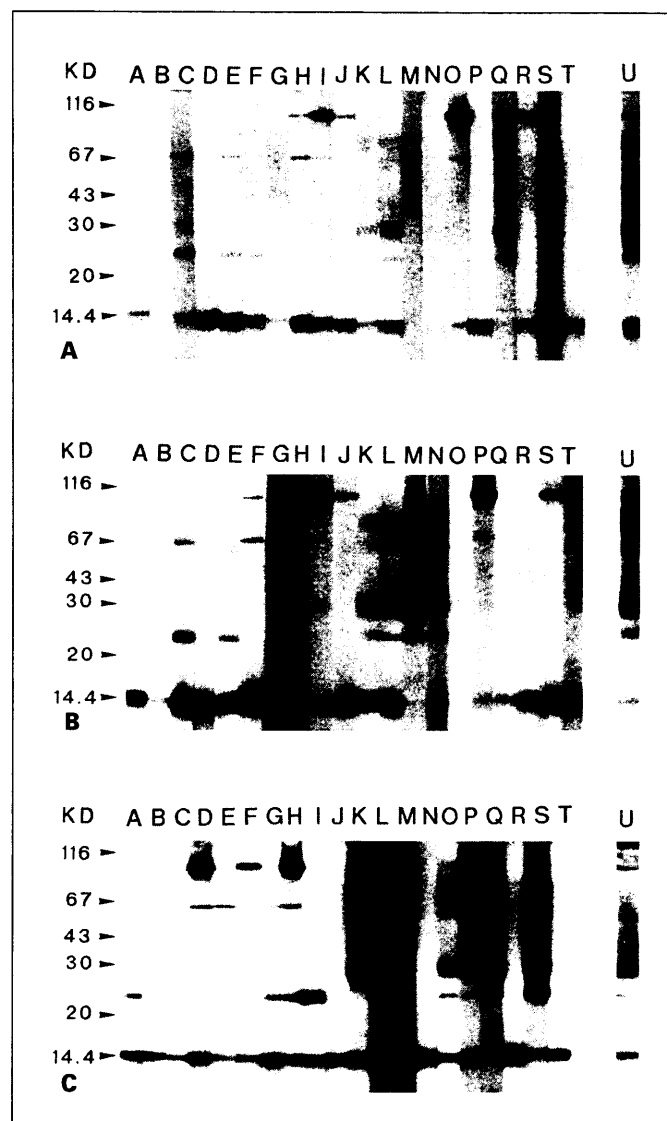


Fig. 1. Comparison of IgE binding of individual sera to Df body extracts between the three groups of children: children with asthma (A), children with AD (B) and children with both asthma and AD (C). Lanes A–T represent 20 individual sera from Df-sensitive atopic children, lane U positive control serum pooled from 3 Df-sensitive asthmatic subjects. The sample sera were adjusted to have the same RAST titer (12.8), and applied to the immunoblot analyses.

The immunoblot study has shown that Der f2 have a higher IgE-binding activity than Der f1 in all three groups. This is consistent with the study of Ford et al. [18], who showed that the Der p 2 allergen had the highest IgE binding activity of any of the major *D. pteronyssinus* allergens including Der p 1 and Der p 3. Since the Df extracts used contained almost equal amounts of Der f1 and Der f2 allergens, it is unlikely that the lower positivity for Der f1 in the immu-

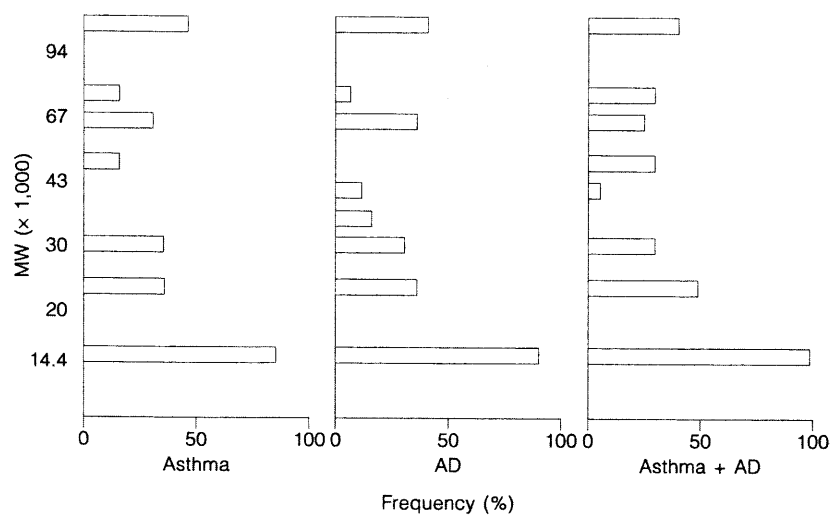


Fig. 2. Frequencies of positive IgE binding reactions of Df body extracts in the three groups of children.

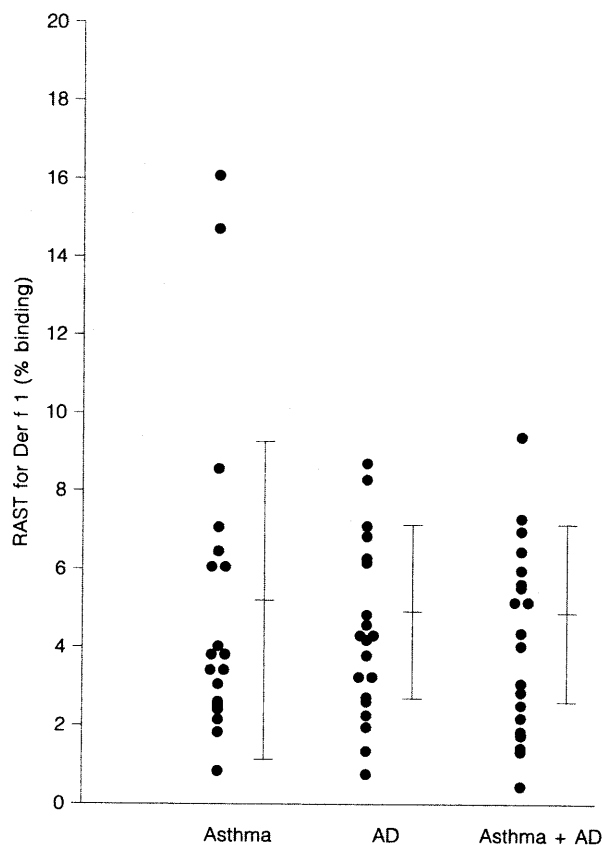


Fig. 3. Comparison of relative RAST reactivity to Der f1 in the three groups of children. The differences in RAST binding between the three groups were not significant ($p > 0.1$) by Mann-Whitney test.

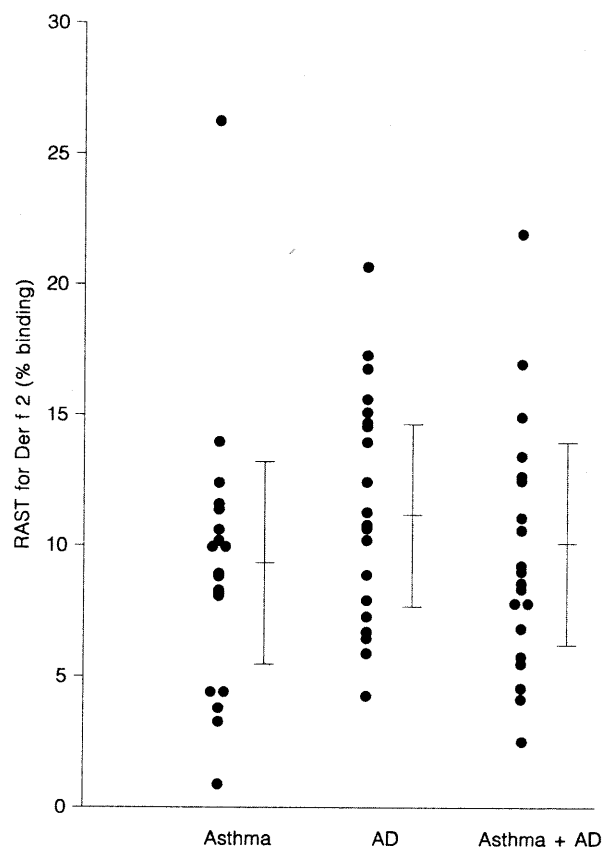


Fig. 4. Comparison of relative RAST reactivity to Der f2 in the three groups of children. The differences in RAST binding between the three groups were not significant ($p > 0.1$) by Mann-Whitney test.

noblot study than in RAST is due to a difference in the loading doses of the two allergens on electrophoresis. On the other hand, it has been shown that Der 1 allergens are more easily denatured than Der 2 allergens by heating, acid treatment, reduction and alkylation [26, 27]. In this study, although Df allergens were neither heated nor acidified, and were electrophoresed under nonreducing conditions, the possibility remains that SDS-PAGE or electric transfer may have degraded the Der f1 allergen.

Carswell and Thompson [28] and Thompson et al. [19] have reported that the concentration of IgE antibodies to Der 2, which mainly originate from mite body antigens, is higher in children with AD than in children with asthma, whereas the concentration of IgE antibodies to Der 1, the fecal allergen, is higher in children with asthma, suggesting that the route of allergic sensitization may be an important factor for distinguishing asthma from AD. On the other hand, Sakaguchi et al. [29] have recently reported that Der 1 concentration determined as an airborne allergen is 3–5 times higher than Der 2, while flour dust samples have an almost equal Der 1 and Der 2 concentration. Our results showing that both asthmatic and AD patients have a higher

IgE-binding activity to Der 2 than Der 1 on immunoblot and a similarly high IgE reactivity to Der 1 and Der 2 in RAST are inconsistent with these previous observations.

The cutaneous lymphocyte-associated antigen (CLA), a major T cell ligand for the vascular adhesion molecule E-selectin, has been proposed to be involved in the selective targeting of memory T cells reactive to skin-associated antigens in cutaneous inflammatory sites. Recently, Babi et al. [30] have demonstrated that restriction of the T cell-proliferative response to a CLA+ memory subset is observed in atopic eczema alone, whereas a CLA- subset exhibits a strong antigen-dependent proliferation in patients with asthma. Although our results cannot be compared with these immunological studies, it seems likely that intrinsic factors, such as a local expression of adhesion molecules in the skin, may be associated with the disease specificity for asthma or AD.

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Images in Allergy and Immunology

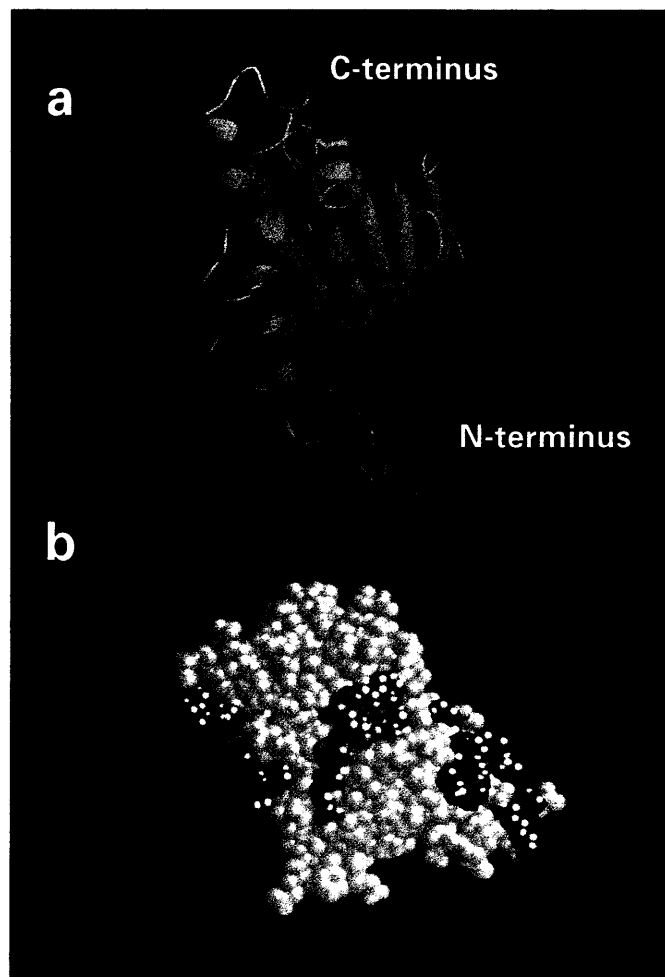
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Structure and Epitopes of Bet v 1

The binding of human IgE to a foreign allergen represents a crucial step in the development of clinical symptoms of type I allergy. The binding of IgE occurs to B-cell epitopes composed of amino acid residues located on the molecular surface of the allergen. Detailed knowledge on the three-dimensional structure of the allergen is thus essential in order to identify and characterize its IgE-binding B-cell epitopes.

a Ribbon representation of the three-dimensional molecular structure of Bet v 1, the major allergen of birch pollen, which has recently been solved both in solution by NMR spectroscopy and in the crystalline state by X-ray crystallography [1, 2]. The main feature of the structure is a seven-stranded anti-parallel β -sheet (orange) that wraps around a 25-residue-long C-terminal α -helix (red). The β -sheet and the C-terminal part of the long helix are separated by two consecutive α -helices. The structure reveals a most unusual feature, a large cavity that penetrates the entire protein with three openings on the molecular surface [2]. **b** The molecular surface of Bet v 1 is visualized by a space-filling model with surface-exposed amino acid residues in color. The areas in green, blue and red represent regions of exposed amino acid residues that are conserved among the major tree pollen allergens of *Fagales*. These areas are likely to harbor cross-reactive B-cell epitopes and provide a structural basis for the clinically observed allergic symptoms that birch-pollen-allergic patients experience when they encounter pollens from related trees such as hazel, alder and hornbeam. The structural determination represents a new and major step towards identifying and modifying IgE-binding B-cell epitopes of the major tree pollen allergens and will be invaluable to the future development of therapy and diagnosis of allergic diseases.



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